

## Effects of drought stress and high density stem inoculations with *Leptographium wingfieldii* on hydraulic properties of young Scots pine trees

LUC CROISÉ,<sup>1,2</sup> FRANÇOIS LIEUTIER,<sup>2,3</sup> HERVÉ COCHARD<sup>1,4</sup> and ERWIN DREYER<sup>1,5</sup>

<sup>1</sup> INRA-Nancy, UMR INRA-UHP Ecologie et Ecophysiologie Forestières, F-54280, Champenoux, France

<sup>2</sup> INRA-Orléans, Station de Zoologie Forestière, F-45160, Ardon, France

<sup>3</sup> Université d'Orléans, Laboratoire de Biologie des Ligneux, BP 6759, 45067 Orleans Cedex, France

<sup>4</sup> Present address: INRA-PIAF, Domaine de Crouelle, 63039 Clermont-Ferrand Cedex, France

<sup>5</sup> Author to whom correspondence should be addressed

Received April 10, 2000

**Summary** We examined drought-induced changes in susceptibility of potted Scots pine (*Pinus sylvestris* L.) trees to a bark-beetle associated fungus (*Leptographium wingfieldii* Morelet, from the bark beetle *Tomicus piniperda* L.). Five-year-old field-grown trees were transplanted to 50-l pots and grown for 1 year before the treatments were applied. Trees in the drought treatment were subjected to several successive, 3-week-long drought cycles, with predawn water potential dropping below  $-2$  MPa at peak drought intensity. The experimental drought cycles were more severe than the natural drought episodes usually recorded in Scots pine stands. Trees were then mass-inoculated with *L. wingfieldii* at a density close to the critical threshold density of inoculations ( $400\text{ m}^{-2}$ ) above which tree resistance is overcome. Inoculation of well-watered trees resulted in induced reaction zones around the inoculation points and very limited damage (resinosis) in the sapwood. Drought alone had no long-lasting consequences on tree water relations, except for a decrease in hydraulic conductance in the youngest segments of the main stem. However, the combination of mass-inoculation and drought stress after inoculation resulted in a dramatic loss of stem hydraulic conductivity that was paralleled by conspicuous damage to the sapwood (resinosis, drying and blue staining). There was a close correlation between amount of visible sapwood damage and loss of hydraulic conductivity. The intensity of induced reactions in the phloem was unaffected by drought stress. We conclude that tree defence against *L. wingfieldii* is decreased during severe drought stress, resulting in changes in the spread and action of the fungus in the sapwood but not in the phloem.

**Keywords:** bark beetles, blue stain fungi, cavitation, phytopathogens, sapwood, tree defence.

### Introduction

Bark beetles (Coleoptera, Scolytidae) are among the most

damaging pests in forests (Berryman 1972, Lieutier and Lévieux 1985). Successful invasion of the phloem and xylem by a bark beetle population may lead to death of invaded trees within a few months. In addition, bark beetles sometimes carry fungi that may play a role in killing trees after beetle attacks. Damage by pine beetles and their associated fungi in forest stands has been reported to increase during years of drought (Hopping and Mathers 1945, Kalkstein 1981). Drought is thought to increase the susceptibility of trees to insect attack; however, the mechanisms and extent of drought-induced changes in tree susceptibility remain a matter of conjecture.

Susceptibility of trees to attack by bark beetles and their associated fungi is generally related to the extent of constitutive resinosis (Lorio and Sommers 1986), and of induced reaction zones in the phloem around inoculation points (Raffa and Berryman 1983, Stephen et al. 1983, Lieutier et al. 1993, Paine et al. 1997). The latter changes with tree age and season (Stephen and Paine 1985, Långström et al. 1992) and may not always adequately reflect a tree's defence ability (Croisé et al. 1998b, Krokene and Solheim 1998).

Drought-induced changes in tree susceptibility to attacks by bark beetles and their associated fungi have been widely documented. Croisé and Lieutier (1993) observed only limited changes in the induced response (local necroses and accumulation of phenolics) in the bark of young Scots pine seedlings subjected to severe drought and inoculations with *Ophiostoma brunneo-ciliatum* Math. and *Leptographium wingfieldii* Morelet. Similarly, Croisé et al. (1998b) recorded only small changes in the dimensions and phenolic composition of induced reactions to *Ophiostoma ips* (Rumb.) Nannf. in severely drought-stressed Scots pine trees. Christiansen (1992) detected no effect of drought preconditioning on the extent of damage induced by massive inoculation of *Ceratocystis polonica* (Siem.) C. Moreau into the trunk of adult *Picea abies*. In contrast, Christiansen and Glosli (1996) reported that *Ceratocystis polonica* caused less blue staining in the trunk

and less phloem damage in moderately stressed trees than in well-watered trees. Similarly, Dunn and Lorio (1993) concluded that drought slightly increased the resistance of *Pinus taeda* L. to *Dendroctonus frontalis* Zimm. Reeve et al. (1995) concluded that defence capacity increased with moderate drought and decreased at severe drought stress intensities.

Responses of trees to massive fungal inoculations in the trunk may be quantified by the resulting death of trees, which may take several months to occur, or the extent of induced reaction zones in the bark, or of damage in the sapwood. The latter is usually expressed as the fraction of cross-sectional sapwood area displaying either induced resinosis, or blue staining caused by the presence of fungal mycelia (Christiansen 1985, Solheim et al. 1993, Croisé et al. 1998a). Although sapwood damage is presumably correlated with important xylem dysfunctions, the effects of massive fungal inoculations on hydraulic conductivity have seldom been measured.

Fungal spread and mycelial growth into sapwood cause localized damage to tracheid cell walls resulting in air seeding into the water columns leading to cavitation events and embolism (Zimmermann 1983). Subsequent xylem blockage by gums, resin deposits and tyloses may render this initial embolism irreversible. Studies of reductions in hydraulic conductivity resulting from cavitation and subsequent embolism in response to drought and winter freezing have shown that cavitation events occur whenever xylem sap tensions increase above a species-specific threshold (Tyree and Sperry 1989, Wei et al. 1999).

The aim of the present work was to test for potential interactive effects between fungal invasion and severe drought stress in inducing cavitation in the xylem of Scots pine trees. We used a fungal species associated with the common pine shoot beetle *Tomicus piniperda* L. This insect is a secondary bark beetle that occasionally kills weakened trees but lacks aggregation pheromones that would help concentrate the attacks on a few individual trees and kill them readily (Långström et al. 1992). The beetle feeds on young shoots of Scots pine (*Pinus sylvestris* L.) during summer, and attacks trunks during spring to lay eggs and reproduce. Långström et al. (1992) found a density threshold of about 500 egg galleries  $m^{-2}$  above which adult trees were killed. This beetle sometimes carries the fungus *Leptographium wingfieldii* (Morelet 1988) that may play a role in killing trees after beetle attacks, but does not seem to interfere with beetle population establishment in the trees (Lieutier et al. 1989, Solheim and Långström 1991, Lieutier 1995). Massive inoculations with *L. wingfieldii* alone may promote conspicuous damage, including blue staining of sapwood and induced resinosis, and even tree death (Långström et al. 1993). Blue staining occurred only above a threshold of 400–800 inoculation points  $m^{-2}$  over a 60-cm wide band and vigorous trees died when inoculated at a density of 800 points  $m^{-2}$ , whereas severely pruned trees were killed by an inoculation density of 400  $m^{-2}$  (Croisé et al. 1998a). Among the fungi associated with bark beetles, *L. wingfieldii* is rather virulent, similar to *Ophiostoma minus* (Hedgcock) Sydow, and more

aggressive than *Ophiostoma brunneo-ciliatum* or *O. ips* (Lieutier et al. 1990).

We subjected young potted Scots pine trees to several successive cycles of severe drought and mass-inoculated them with a strain of *L. wingfieldii*, in a cross-factorial experimental design. The extent of damage and loss of hydraulic conductivity in the sapwood were recorded 3 months after inoculation. In addition, we tested whether repeated drought cycles before inoculation had any additive effects on this sensitivity.

## Material and methods

### Plant material and growth conditions

In January 1992, 48 five-year-old Scots pine (*Pinus sylvestris* L.) trees growing in a nursery near Mâcon, France, were selected for homogeneity in height (1.25 m), stem diameter, and foliage density. The trees were lifted with their soil ball during February 1992, planted in 50-l pots filled with a 2:1 (v/v) sand:peat mixture, and grown outside in a nursery near Nancy, northeastern France. During April 1992, all trees were supplied with a slow-release fertilizer (Nutricote 100, N,P,K 13:13:13, + oligoelements, 250 g tree<sup>-1</sup>). One year after transplanting, the potted trees were moved to a greenhouse (temperature: 12–25 °C; relative humidity: 50–95%; transmitted irradiance: two-thirds of that outside). A white plastic cover was wrapped around each pot to minimize direct soil evaporation.

### Experimental design

Sixteen trees were randomly assigned to each of Groups I, II and III in spring 1993, and four treatments were applied to each group of trees. The treatments were: (1) water stressed and inoculated with *Leptographium wingfieldii* ( $n = 6$ ); (2) water stressed and non-inoculated ( $n = 2$ ); (3) well watered and inoculated with *L. wingfieldii* ( $n = 6$ ); and (4) well watered and non-inoculated ( $n = 2$ ). The well-watered control trees were watered twice a week to field capacity throughout the experiment (April–October 1993), whereas drought-stressed trees were subjected to an increasing number of 12–50-day drought cycles (3, 5 and 7 drought cycles for trees in Groups I, II and III, respectively). During each drought cycle, trees were left to dry by withholding irrigation until a needle water potential ( $\Psi_{wp}$ ) expected to result in complete stomatal closure was reached (around  $-1.8$  MPa). The trees were then re-watered to field capacity before the next drought cycle. For 2 months after inoculation, drought stress was stabilized at a predawn  $\Psi_{wp}$  of about  $-1.5$  MPa by adding 300 to 700 ml of tap water every second day according to climatic demand.

Predawn needle water potential was measured weekly or every second week beginning at the end of April 1993. In addition, during the third drought cycle, we recorded net assimilation rate ( $A$ ), stomatal conductance ( $g_s$ ), and soil volumetric water content ( $H_v$ ) of potted trees of Group I (see water relations). Needle biomass was estimated for each branch whorl. Trunk diameter, and hydraulic conductivity ( $K_t$ ) were measured on all trees at the end of the experiment.

### Inoculation with fungus

*Leptographium wingfieldii* was collected from bark-beetle galleries on Scots pine in the forest of Orleans, France, and grown on a malt agar medium as a monospore strain. At each inoculation point, a plug of bark and phloem was removed with a cork borer, and a 5-mm diameter disk of a 3-week-old malt agar culture was inserted, with the fungus close to the sapwood. Thereafter, the bark plug was replaced to maintain the mycelium, and to avoid contamination. Inoculation density was 400 m<sup>-2</sup> on 90 cm of stem (Figure 1). Inoculations were distributed on several successive staggered rings to avoid systematic coalescence of the induced reaction zones in the phloem and sapwood. The last stem segments (grown during 1992 and 1993) were kept free from any inoculation. Inoculation schedule was as follows: June 30 (Day 181, beginning of the third drought cycle) for Group I; August 2 (Day 214, beginning of the fifth drought cycle) for Group II; August 26 (Day 238, beginning of the seventh drought cycle) for Group III. Ten weeks after inoculation, trees were harvested for damage assessment, hydraulic conductivity measurements and biomass (September 13–28, Days 256–271 for Group I; October 21–29, Days 294–302 for Group II; and November 3–11, Days 307–315 for Group III).

### Water relations

Soil volumetric water content was estimated in Group I trees once a week before re-watering, during the third drought cycle, by time domain reflectometry (TDR; Soil Moisture Equipment Corp., Santa Barbara, CA) with 40-cm-long wave guides. Relative extractable soil water (REW) was computed as:

$$\text{REW} = (R - R_{\min}) / (R_{\max} - R_{\min}),$$

where  $R$ ,  $R_{\max}$  and  $R_{\min}$ , are actual, maximal (e.g., at field capacity) and minimal (e.g., at the end of the drought cycles) soil water contents (mm), respectively. The value of  $R_{\max} - R_{\min}$  (which is an estimate of the extractable soil water) was close to 12.5 l in the 50-l pots.

Predawn ( $\Psi_{\text{wp}}$ , MPa) and midday ( $\Psi_{\text{m}}$ , MPa) needle water potentials were measured every second week with a pressure chamber on a single, current-year needle on the 1992 whorls. Stomatal conductance to water vapor ( $g_s$ , mmol m<sup>-2</sup> s<sup>-1</sup>) was measured during sunny days (between Days 180 and 250 for Group I) at midday with a portable gas exchange system (LI-6200, Li-Cor, Lincoln, NE) on one shoot per plant at the 1992 whorl level. Soil-to-leaf specific hydraulic conductance ( $g_L$ , mmol m<sup>-2</sup> s<sup>-1</sup> MPa<sup>-1</sup>) was calculated according to Cohen et al. (1983) and Reich and Hinckley (1989) from the relationship:

$$g_L = E / (\Psi_{\text{wp}} - \Psi_{\text{m}}),$$

where  $E$  is transpiration flux density (mmol m<sup>-2</sup> s<sup>-1</sup>). Transpiration was estimated from the loss in weight of potted trees during a 10-min period between 1100 and 1300 h, and expressed on a needle area basis. Soil evaporation was considered to be negligible, because of the white plastic covers and the large needle area. Total needle area ( $NA_t$ , m<sup>2</sup>) was estimated by means of a calibration curve established with the same trees:

$$NA_t = (\text{DW}67.2) / 10^4,$$

where DW is needle dry weight (g, 24 h at 80 °C). Developed needle area was calculated from projected needle area of sample needles ( $NA_p$ ) measured with an area meter (Delta-T Devices, Burwell, England). Needle cross section was assumed to be hemi-cylindrical, and total needle area was calculated as:

$$NA_t = NA_p 2.57.$$

Hydraulic conductivity ( $K_i$ ; kg m s<sup>-1</sup> MPa<sup>-1</sup>) was measured in all trees 2.5 months after inoculation following the technique of Sperry et al. (1988) as described by Cochard (1992) and Mencuccini and Grace (1996) for Scots pine. The  $K_i$  was measured in three segments of different ages (axis grown during 1988, 1990 and 1992; see Figure 1) from the main stem. The youngest segment (1992) had not been subjected to inoculation. Trees were cut at the root collar, and transported to a laboratory; where measurements were conducted at 20 ± 3 °C. Samples about 10-cm long were recut under water, and the two cut ends recut with a razor blade to avoid occlusion of the tracheids by resin exudates. Hydraulic conductivity was measured with distilled and degassed water containing fast-green dye (Sigma, St. Louis, MO) by applying a constant water pressure of 5 kPa, and by measuring the resulting water flux through the samples:

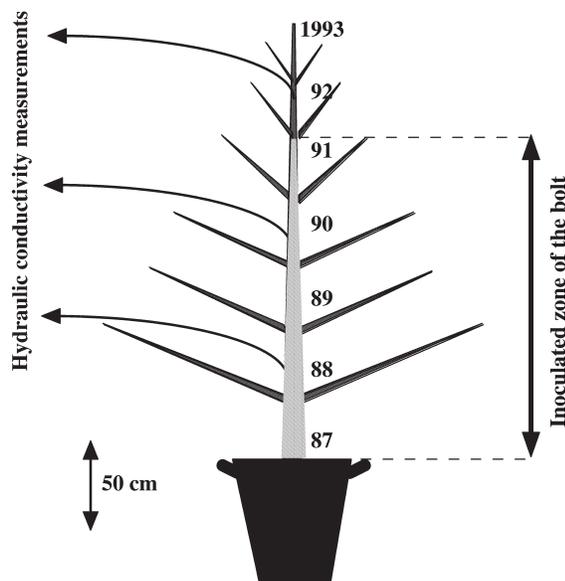


Figure 1. Diagram of the distribution of inoculation points and main-stem segments collected for measurement of hydraulic conductivity on Scots pine trees. Numbers adjacent to the stem represent the year of formation of the successive stem segments.

$$K_i = (FL)/P,$$

where  $F$  is flux through the segment ( $\text{kg s}^{-1}$ );  $L$  is segment length (m) and  $P$  is applied pressure (MPa). This step was performed less than half an hour after collecting the samples to avoid passive resaturation of the xylem. Fast green was used because of its ability to diffuse along non-embolized tracheids. Maximal hydraulic conductivity  $K_m$  was not measured directly on the same segments, because of the potential occurrence of occlusions in xylem vessels after long-term stress and fungal invasion. Instead, we calibrated a relationship between  $K_i$  and the diameter of non-inoculated segments on well-watered control trees and used it to estimate  $K_m$  of the other segments (Figure 2). The degree of embolism was then computed as percent loss of conductivity (PLC):

$$\text{PLC} = 100(K_m - K_i)/K_m.$$

Because of the scatter in the  $K_m$ -diameter relationship, this computation procedure sometimes resulted in negative values. Leaf-specific conductivity (LSC) was estimated as:

$$\text{LSC} = K_i/\text{LA},$$

where LA is total needle area distal to the stem segment ( $\text{m}^2$ ).

Perfused samples were collected, recut at the level of inoculation points and the fractions of cross-sectional area either (1) stained by fast green dye, i.e., functional sapwood, (2) resin-soaked, (3) dried or (4) blue-stained by the fungus were computed after digitizing and image analysis.

#### Assessment of induced reaction zone surface in the phloem

Two and a half months after inoculation, the outer bark was removed around each inoculation point for trees of Group III,

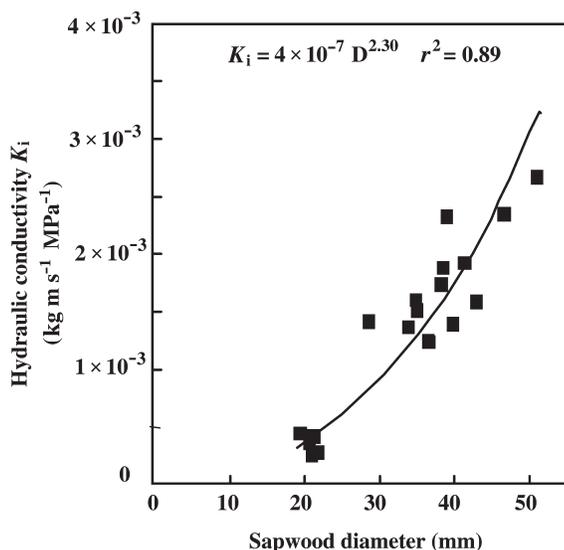


Figure 2. Initial hydraulic conductivity ( $K_i$ ) versus sapwood diameter recorded on well-watered and non-inoculated Scots pine trees. Each value represents one stem segment ( $n = 3$  segments  $\times 6$  trees).

and the area of induced reaction zones was measured on the external side of the phloem.

#### Data analysis

Statistics were performed with SAS software after data had been tested for normality (SAS Institute, Cary, NC). Analysis of variance (GLM procedure) or the Student  $t$ -test was used to compare treatments, and the level of significance was tested at  $P < 0.05$ . We used the repeated-measures method to make comparisons between dates for a given parameter, each parameter was always measured on the same seedling. Bars in the figures represent  $\pm$  SE.

## Results

### Time course of drought

Well-watered trees exhibited stable  $\Psi_{wp}$  values throughout the experiment ( $\Psi_{wp} = -0.4 \pm 0.01$  MPa, Figure 3). Withholding water induced sharp and fast decreases in  $\Psi_{wp}$  to between  $-2.5$  and  $-3.2$  MPa in about 3 weeks. After re-watering to field capacity, and despite the severe drought,  $\Psi_{wp}$  always recovered rapidly to values close to those of control trees. Inoculation with *L. wingfieldii* had no visible effect on the time course of  $\Psi_{wp}$  for either well-watered or drought-stressed trees. During inoculation and at harvest,  $\Psi_{wp}$  was significantly lower in drought-stressed trees than in well-watered trees of Groups II

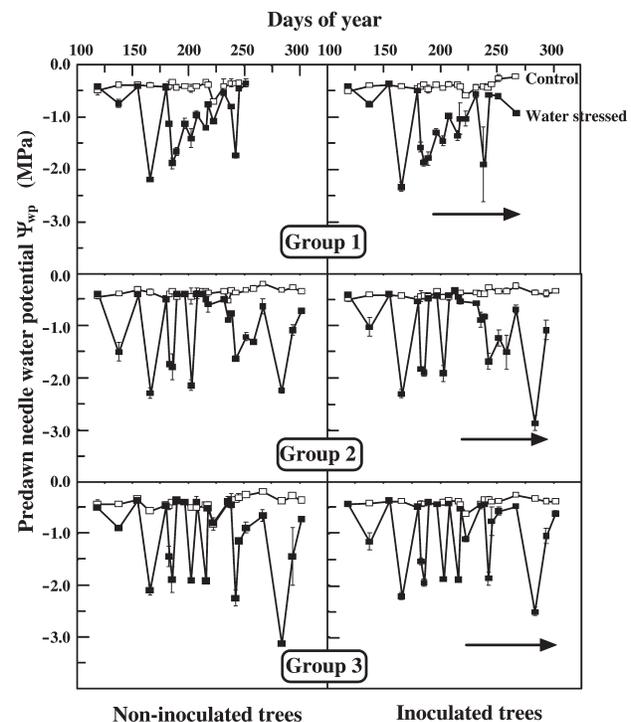


Figure 3. Time course of predawn needle water potential ( $\Psi_{wp}$ ) in control ( $\square$ ) and drought-stressed ( $\blacksquare$ ) Scots pine trees. Values are means  $\pm$  SE ( $n = 6$ : inoculated and 2: non-inoculated). Inoculations were made on Days 181, 214 and 238 for trees in Groups I, II and III, respectively. Arrows indicate the time from inoculation to harvest.

and III but not of Group I (Table 1). However, mean  $\Psi_{wp}$  computed for the 2–5-month period from inoculation to harvest was always significantly lower in drought-stressed trees than in controls.

#### Soil water availability

In well-watered pots, soil volumetric water content ( $H_v$ ) varied between 25 and 10% in the 2–3-day interval between two successive irrigations, with no difference between inoculated and non-inoculated trees. Withholding irrigation resulted in a sharp decrease in  $H_v$  to 4%. Relative extractable water never decreased below 0.4 in well-watered pots, but was reduced to 0.0–0.2 in pots in the drought treatment at the end of the drought cycles (Table 2).

#### Growth

At the end of the experiment, all trees were about 2 m high with a basal diameter of about 6 cm. Because the drought treatment was imposed at the end of the phase of main axis elongation, it did not reduce height growth (Table 3). However, basal stem diameter increment was less in drought-stressed trees than in control trees. Drought resulted also in a significant reduction in needle biomass in trees in Group I, but not in trees in Groups II and III. The reduction in needle biomass was caused

by enhanced senescence of older needles and not by limitations of needle growth, which had already stopped at the onset of drought. Inoculation alone had no detectable impact on either height growth or diameter increment.

#### Water relations

Water relations were monitored daily on four trees in Group I during the third drought cycle. Well-watered, non-inoculated trees exhibited stable values of  $\Psi_{wp}$  and  $\Psi_m$  (mean values of  $-0.44$  and  $-1.16$  MPa, respectively). Soil water content ( $H_v$ ) was 18.9%, representing a total reserve of 9.1 l, and relative extractable water was 0.58 (Table 2). Stomatal conductance ( $g_s$ ) varied between 50 and 80  $\text{mmol m}^{-2} \text{s}^{-1}$  (data not shown). Mean soil-to-needle specific hydraulic conductance ( $g_L$ ) was about  $1.0 \text{ mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ . Whole-plant transpiration on a needle area basis was about  $0.7 \text{ mmol m}^{-2} \text{s}^{-1}$  at midday and varied little during this period. Inoculations with *L. wingfieldii* had no effect on any of these parameters (Table 2).

The drought-induced decline in water potential was accompanied by large reductions in whole-tree transpiration (Table 2) and  $g_s$  (data not shown). Similarly,  $g_L$  declined to around  $0.2 \text{ mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$  (Table 2). All of these parameters recovered after the pots were re-watered to field capacity (during Day 245). No effect of inoculation was detected on any of

Table 1. Predawn needle water potentials of Scots pine trees at inoculation and at harvest, and mean value during the 2.5 months between inoculations and harvest. Because inoculation had no effect on predawn needle water potential, inoculated and non-inoculated trees were pooled into control and water-stress treatments. Values are means  $\pm$  SE. Within rows, and inside each Group, different letters indicate significant differences ( $P < 0.05$ , ANOVA followed by Bonferroni test,  $n = 8$ ).

Time of observation	Predawn needle water potential (MPa)					
	Group I		Group II		Group III	
	Control	Drought	Control	Drought	Control	Drought
At inoculation	$-0.45 \pm 0.02$ a	$-0.47 \pm 0.02$ a	$-0.39 \pm 0.02$ a	$-0.47 \pm 0.02$ b	$-0.36 \pm 0.03$ a	$-0.45 \pm 0.02$ b
At harvest	$-0.30 \pm 0.05$ a	$-0.39 \pm 0.02$ a	$-0.36 \pm 0.05$ a	$-1.10 \pm 0.14$ b	$-0.38 \pm 0.03$ a	$-0.65 \pm 0.04$ b
Mean for period between inoculation and harvest	$-0.42 \pm 0.02$ a	$-1.41 \pm 0.18$ b	$-0.36 \pm 0.02$ a	$-1.08 \pm 0.04$ b	$-0.34 \pm 0.01$ a	$-1.11 \pm 0.06$ b

Table 2. Soil water content ( $H_v$ ), available soil water per pot, relative extractable water (REW), predawn and midday needle water potential ( $\Psi_{wp}$  and  $\Psi_{wm}$ ), transpiration ( $E$ ) and soil to needle specific hydraulic conductance ( $g_L$ ) recorded during the third cycle of drought for two Scots pine trees per treatment (means of two trees  $\times$  seven dates).

Parameter	Control non-inoculated	Control inoculated	Drought non-inoculated	Drought inoculated
<i>Soil</i>				
$H_v$ (%)	18.9	18.2	6.8	6.8
Available water (liter)	9.1	8.7	3.3	3.3
REW	0.58	0.55	0.11	0.11
<i>Plants</i>				
$\Psi_{wp}$ (MPa)	$-0.44$	$-0.48$	$-1.23$	$-1.24$
Mean $\Psi_{wm}$ (MPa)	$-1.16$	$-1.19$	$-1.66$	$-1.83$
Minimum $\Psi_{wm}$ (MPa)	$-1.38$	$-1.70$	$-2.10$	$-2.18$
$E$ ( $\text{mmol m}^{-2} \text{s}^{-1}$ )	0.66	0.58	0.17	0.20
$g_L$ ( $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ )	1.02	0.86	0.41	0.30

Table 3. Effects of drought on height, diameter of main axis and needle biomass of Scots pine trees during the fifth year of growth. Values are means  $\pm$  SE. Different letters indicate significant differences ( $P < 0.05$ , ANOVA followed by Bonferroni test,  $n = 8$ ), between drought-treated and control trees. Groups differ by the number of drought cycles imposed earlier (see text).

Parameter	Group I		Group II		Group III	
	Control	Drought	Control	Drought	Control	Drought
Height (cm)						
Total	198 $\pm$ 5.5 a	200 $\pm$ 5.7 a	211 $\pm$ 3.5 a	202 $\pm$ 4.0 a	199 $\pm$ 5.0 a	199 $\pm$ 2.0 a
Leading shoot, 1993	48 $\pm$ 2.8 a	50 $\pm$ 2.4 a	60 $\pm$ 2.9 a	52 $\pm$ 4.5 a	56 $\pm$ 1.6 a	50 $\pm$ 1.3 a
Basal diameter (cm)	6.3 $\pm$ 0.2 a	5.6 $\pm$ 0.2 b	7.1 $\pm$ 0.7 a	5.6 $\pm$ 0.2 b	5.6 $\pm$ 0.1 a	5.3 $\pm$ 0.2 a
Needle biomass (DW, g)						
Total	1055 $\pm$ 47 a	767 $\pm$ 39 b	745 $\pm$ 66 a	755 $\pm$ 36 a	705 $\pm$ 56 a	633 $\pm$ 32 a
Whorl, 1993	98 $\pm$ 18 a	93 $\pm$ 7 a	99 $\pm$ 14 a	73 $\pm$ 7 a	92 $\pm$ 9 a	75 $\pm$ 3 a

these reversible, drought-induced reductions.

#### *Induced reaction zones in the phloem*

The area of the induced reaction zones, which was measured on trees in Group III, was not affected by either drought stress or height on the trunk (Table 4). It was close to 2.5–3 cm<sup>2</sup> in all cases.

#### *Embolism in stem segments and damage to sapwood*

Two trees in the drought + inoculated treatment (among the 18 trees in this treatment) exhibited blue staining over about 85% of the sapwood surface at ground level, and concomitant dark brown coloration of the whole phloem in the inoculated stem (1992 and 1993 growth). Predawn needle water potential ( $\Psi_{wp}$ ) started to decrease sharply from Day 220 (i.e., 39 days after inoculation) and reached values of  $-4.0$  MPa at the end of the third drought cycle. Leaf specific hydraulic conductivity (LSC) of the 1988 growth segment was near zero.

Because no difference related to the number of drought cycles imposed before inoculation was detected in any of the tested parameters, trees from Groups I, II and III were pooled for the statistical analysis. The LSC recorded on stem segments revealed several features (Figure 4). (1) The LSC increased from stem base to top; (2) it responded to drought only in the youngest trunk segments (with a 50% loss of conductivity in the 1992 segment); (3) it was not affected by massive in-

oculations in either 1988 or 1990 growth segments; (4) it decreased significantly only in response to the combination of drought and massive inoculations; and (5) it declined markedly in the non-inoculated 1992 segment of inoculated trees. Percent loss of conductivity did not significantly differ from 0 in the oldest (1988 and 1990) stem segments of control and drought-stressed trees (Figure 5). The combination of drought + inoculation resulted in high mean PLC values (around 50%), and a few individuals displayed PLC close to 100%. In contrast, the youngest segments (1992) displayed significant PLC in both inoculated and non-inoculated trees in the drought treatment.

The extent of sapwood damage, recorded as the fractions of blue-stained, resin-soaked or dried, sapwood cross-sectional area also revealed marked treatment responses (Figure 5). Blue staining occurred only in the inoculated segments (1988 and 1990) of drought-stressed trees. Resin soaking was observed only in the inoculated segments of drought-stressed and well-watered trees, and was of similar extent in both cases. Dried sapwood represented a large fraction of the cross section in the inoculated and drought-stressed trees (1988 and 1990 segments) but represented only a small fraction of the cross section of all other segments (10%). The non-inoculated 1992-grown section of the stems presented only sapwood drying, with similar amounts in all treatments (cross-sectional fraction of dried sapwood was  $7.7 \pm 1.6$ ,  $6.3 \pm 1.0$ ,  $5.9 \pm 0.8$ , and  $4.8 \pm 0.8\%$ , in inoculated + drought-stressed, inoculated + well-watered, non-inoculated + water-stressed, and non-inoculated and well-watered trees, respectively).

Loss of functional sapwood cross section and percent loss of hydraulic conductivity were closely correlated, despite a large scatter of data at low levels of damage (due to PLC computing procedure; Figure 6). The overall correlation was significant ( $r^2 = 0.77$ ) and was even higher for inoculated + drought-stressed trees ( $r^2 = 0.88$ ), but it was not significant for inoculated + well-watered trees ( $r^2 = 0.27$ ).

## Discussion

The interaction between drought stress and plant susceptibility

Table 4. Effect of drought on area (cm<sup>2</sup>) of the individual reaction zones induced by inoculating phloem of Scots pine stems with *L. wingfieldii*. Dates indicate the year of development of the corresponding stem segment. Values are means of 3–4 inoculation points  $\times$  six trees per segment. There were no significant differences between years (Bonferroni,  $P = 0.05$ ).

Stem segment	Control	Drought
1991	2.6 a	3.6 a
1990	2.7 a	4.6 a
1989	2.7 a	2.0 a
1988	3.1 a	2.3 a
1987	3.8 a	3.3 a

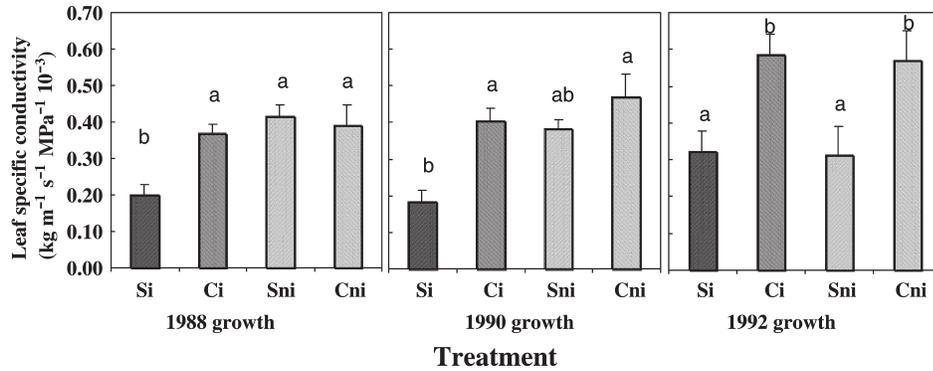


Figure 4. Leaf specific conductivity (LSC,  $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$ ) measured in segments excised from the main stem of Scot pine trees subjected to drought stress and mass inoculated with *Leptographium wingfieldii* in the trunk. The segments were obtained from 1988, 1990 and 1992 growth. Treatments: Cni = control + non-inoculated ( $n = 6$ ); Ci = control + inoculated ( $n = 18$ ); Sni = drought-stressed + non-inoculated ( $n = 6$ ); Si = water-stressed + inoculated ( $n = 16$ ). The 1992 growth segment was not inoculated. Values are means  $\pm$  SE. Within each stem segment age, different letters indicate significant differences ( $P < 0.05$ , ANOVA followed by Bonferroni test).

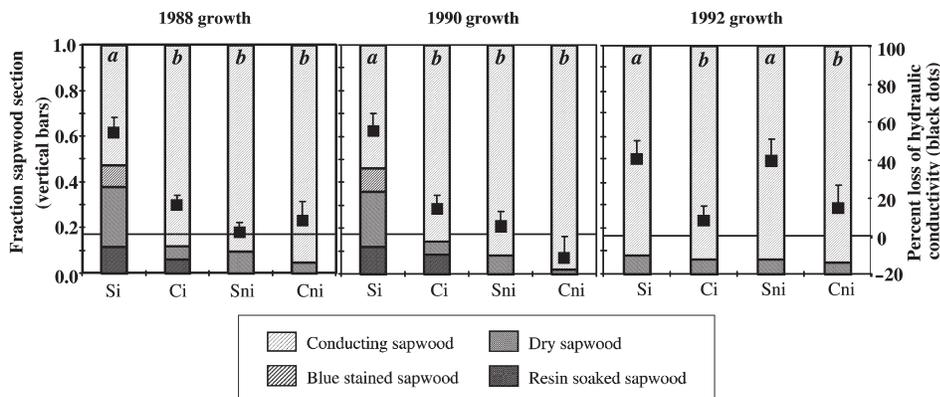


Figure 5. Effects of treatments on the fraction of sapwood cross section still conducting, blue-stained, resin-soaked or dehydrated (vertical bars), and the relative loss of conductivity (black squares) recorded in the same stem segments (black squares, means  $\pm$  SE). The segments were obtained from 1988, 1990 and 1992 growth. The 1992 segment was not inoculated. Treat-

ments: Sni = drought-stressed + non-inoculated ( $n = 6$ ); Si = water-stressed + inoculated ( $n = 16$ ). Different letters indicate differences in functional sapwood cross section in a given stem segment ( $P < 0.05$ ).

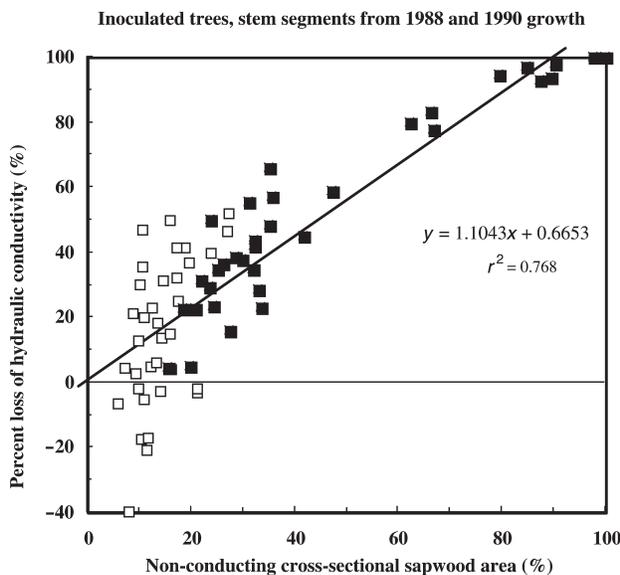


Figure 6. Correlation between loss of conducting sapwood cross section and loss of hydraulic conductivity in stem segments from well-watered (■) and drought-stressed (□) Scots pine trees mass-inoculated with *Leptographium wingfieldii* ( $n = 18$  trees and two segments (1988 and 1992) per tree per treatment).

to herbivores and other insects remains controversial (see review by Waring and Cobb 1992). In the case of bark beetles and their associated fungi, it has been hypothesized that drought modulates resistance, and that constitutive (e.g., oleoresin flow) and induced defence mechanisms respond differentially to environmental constraints (Koricheva et al. 1998). Lombardero et al. (2001) showed that induced oleoresin flow in *Pinus taeda* increased during peak summer drought, whereas constitutive flow was not sensitive to water availability. In the case of bark beetles and their associated fungi, it has been hypothesized that moderate drought increases induced resistance, whereas a severe drought decreases it (Lorio 1986, Lorio et al. 1995, Reeve et al. 1995). We tested the effects of a drought treatment that would be expected to decrease the resistance of Scots pines to the bark-beetle associated fungus complex (*Pinus sylvestris*/*Tomicus piniperda*/*Leptographium wingfieldii*).

Fungal spread into the sapwood may be the cause of severe dysfunctions in water transport. Such dysfunctions, estimated from the loss of hydraulic conductivity, have been observed with a range of pathogens (*Endothia parasitica* (Murrill) P.J. & H.W. Anderson on chestnut, Ewers et al. 1989; *Ophiostoma ulmi* (Buisman) Nannf. on elm trees, Newbanks et al. 1983;

roots of ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) infested with *Leptographium wagneri* (Kendr.) Wingf. and *Heterobasidion annosum* (Fr.) Bref., Joseph et al. 1998). Horntvedt et al. (1983) observed similar decreases with *Ceratocystis polonica* (associated with *Ips typographus* L.) in spruce.

Despite the relative virulence of *Leptographium wingfieldii* compared with other Ophiostomatales associated with bark beetles, mass inoculation of well-watered, potted 5-year-old Scots pines, at a density close to 400 m<sup>-2</sup> had only limited effects 2.5 months after inoculation. It induced the build-up of reaction zones around the inoculation points in the phloem, and caused visible but limited damage in the sapwood, mainly as a result of resinosis. Such responses correspond to the frequently reported symptoms induced by mass inoculation of blue-stain fungi (Christiansen 1985, Croisé et al. 1998b, Krokene and Solheim 1999). Invasion of sapwood is frequently observed but is not a requisite for a successful beetle attack (Paine et al. 1997). The inoculation density of 400 m<sup>-2</sup> was therefore probably below the threshold inoculation density needed to kill vigorously growing young Scots pine trees.

Predawn needle water potential dropped to -2.5 MPa during peak stress, and was kept at about -1.3 MPa for 2.5 months after inoculation. Such drought stress is known to induce almost complete stomatal closure and cessation of net CO<sub>2</sub> assimilation in this species (Jackson et al. 1995, Croisé et al. 1998b). Nevertheless, within a few days after re-watering, predawn needle water potentials of the drought-stressed trees recovered to values similar to those of controls. Although total needle area and height growth were not affected by the drought treatment (because of its late application), the treatment resulted in a significant decrease in stem diameter increment.

Severe drought may cause the onset of cavitation and xylem embolism in Scots pine. Cochard (1992) showed that embolism and loss of hydraulic conductivity began at -2 MPa xylem water potential in this species, and that 50% conductivity was lost below -3 MPa. Water potential in the xylem may differ significantly from that in the needles because of large resistances to water diffusion in needles (Mencuccini and Grace 1996), and because the actual needle potential resulting in xylem cavitation cannot be exactly assessed. A decrease in specific hydraulic conductance, probably due to embolism, was detected in the youngest segment of the main stem, showing that the xylem water potential reached during drought was probably close to the set point of runaway embolism. No visible drought-induced damage was detected in the sapwood.

The combination of drought and mass inoculation did not induce any change in the area of induced reaction zones in the phloem (cf. Croisé et al. 1998b). However, the drought + mass inoculation treatment had dramatic effects in the sapwood. The loss of specific conductivity on a sapwood area basis was around 50% and reached almost 100% in a few individuals that were probably already dying. Loss of specific conductivity was strongly correlated with visible damage in sapwood cross sections. The observed interaction was not a result of a

more severe drought in inoculated trees, because the lowest values of predawn needle water potential were similar in well-watered and drought-stressed trees.

Duration and number of drought periods experienced before inoculation had no impact on tree responses (cf. Christiansen 1992). The observed damage was therefore probably a direct response to drought experienced during inoculation and disease development, and not a cumulative and delayed effect of drought-induced reductions of growth. It has sometimes been assumed that fungal spread follows embolism, and that fungal hyphae are only able to colonize non-functional sapwood. The close correlation between the extent of damage in the sapwood and the loss of conductivity demonstrates that fungal spread was one of the causes, and not solely the consequence of drought-induced embolism.

The fate of trees at this stage of dysfunction is not known. We did not detect any treatment effects on whole-tree water relations before felling. Soil-to-needle, specific hydraulic conductance was affected by the combined treatment, but in a similar way to water stress. An explanation for this is that, in the soil-to-needle pathway, resistance in the sapwood is only minor (about 10–20% of the total resistance) and doubling it would cause only a 20–40% increase in total soil-to-needle resistance. In the longer term, we assumed that trees with the highest sapwood impairment and lowest hydraulic conductivity (PLC close to 100%) would probably die if re-watered.

The occurrence of drought favored the spread of the fungus into the sapwood, where it resulted in enhanced vulnerability to cavitation and decreased xylem water potential (Tyree and Sperry 1989). Although we cannot identify the cascade of events leading to the observed dysfunctions, we can nevertheless conclude that severe drought reduced the trees' capacity for defence against *L. wingfieldii*. In general, the fungus associated with bark beetles is thought to decrease the resistance of the attacked trees and to help successful colonization and digging of egg-galleries (Raffa and Berryman 1983, Christiansen et al. 1987, review by Paine et al. 1997). It is also not known if the drought-induced decrease in resistance to the fungus favors subsequent invasion by bark beetles. Paine et al. (1997) concluded that fungal colonization of sapwood may kill trees, but is not a requisite for successful invasion by insects. The fungus-induced effects that we observed probably occurred too late in the season to be of any help to bark beetles. Moreover, we note that *L. wingfieldii* is only weakly associated with *Tomicus piniperda*, and plays no role in favoring invasion by the insect. However, our observations may be relevant to studies of associations between fungus and beetles producing aggregation pheromones like *Ips acuminatus*–*Ophiostoma brunneo-ciliatum*, or *Ips typographus*–*Ceratocystis polonica*.

#### Acknowledgments

L.C. was supported by a Ph.D. grant of the French Ministry for Higher Education and Research. The authors thank Jean Marie Gioria, who prepared the trees and helped with ecophysiological measurements.

## References

- Berryman, A.A. 1972. Resistance of conifers to invasion by bark beetle–fungi associations. *BioScience* 22:598–602.
- Christiansen, E. 1985. *Ceratocystis polonica* inoculated in Norway spruce: blue staining in relation to inoculum density, resinosis and tree growth. *Eur. J. For. Pathol.* 15:160–167.
- Christiansen, E. 1992. After-effects of drought did not predispose young *Picea abies* to infection by bark beetle-transmitted blue-stain fungus *Ophiostoma polonicum*. *Scand. J. For. Res.* 7:557–569.
- Christiansen, E., R.H. Waring and A.A. Berryman. 1987. Resistance of conifers to bark beetle attack: searching for general relationships. *For. Ecol. Manage.* 22:89–106.
- Christiansen, E. and A.M. Glosli. 1996. Mild drought enhances the resistance of Norway spruce to a bark beetles-transmitted blue-stain fungus. *In Dynamics of Forest Herbivory: Quest for Pattern and Principle*. Eds. W.J. Mattson, P. Niemela and M. Rousi. USDA Forest Service GTR NC-183, pp 192–199.
- Cochard, H. 1992. Vulnerability of several conifers to air embolism. *Tree Physiol.* 11:73–83.
- Cohen, Y., M. Fuchs and S. Cohen. 1983. Resistance to water uptake in a mature citrus tree. *J. Exp. Bot.* 34:451–460.
- Croisé, L. and F. Lieutier. 1993. Effects of drought on the induced defence reaction of Scots pine to bark beetle-associated fungi. *Ann. Sci. For.* 50:91–97.
- Croisé, L., E. Dreyer and F. Lieutier, 1998a. Scots pine responses to increasing numbers of inoculation points with *Leptographium wingfieldii* Morelet, a bark beetle associated fungus. *Ann. Sci. For.* 55:497–506.
- Croisé, L., E. Dreyer and F. Lieutier. 1998b. Effects of drought and severe pruning on the reaction zone induced by single inoculations with *Ophiostoma ips* in the phloem of young Scots pines. *Can. J. For. Res.* 28:1814–1824.
- Dunn, J.P. and P.L. Lorio. 1993. Modified water regimes affect photosynthesis, xylem water potential, cambial growth, and resistance of juvenile *Pinus taeda* L. to *Dendroctonus frontalis*, Coleoptera, Scolytidae. *Environ. Entomol.* 22:948–957.
- Ewers, F.W., P. McManus, A. Goldman, R. Gucci and D. Fulbright. 1989. The effect of virulent and hypo-virulent strains of *Endothia parasitica* on hydraulic conductance in american chestnut. *Can. J. Bot.* 67:1402–1407.
- Hopping, G.R. and W.G. Mathers. 1945. Observations on outbreaks and control of the mountain pine beetle in the lodgepole pine stands of Western Canada. *For. Chron.* 21:98–108.
- Hornthvedt, R., E. Christiansen, H. Solheim and S. Wang. 1983. Artificial inoculation with *Ips typographus*-associated blue-stain fungi can kill healthy Norway spruce trees. *Medd. Nor. Inst. Skogforsk.* 38:1–20.
- Jackson, G.E., J. Irvine and J. Grace. 1995. Xylem cavitation in Scots pine and Sitka spruce saplings during water stress. *Tree Physiol.* 15:783–790.
- Joseph, G., R.G. Kelsey and W.G. Thies. 1998. Hydraulic conductivity in roots of ponderosa pine infected with black stain (*Leptographium wageneri*) or annosus (*Heterobasidion annosum*) root disease. *Tree Physiol.* 18:333–339.
- Kalkstein, L.S. 1981. An improved technique to evaluate climate–southern pine beetle relationships. *For. Sci.* 27:579–589.
- Koricheva, J., S. Larsson and E. Haukioja. 1998. Insect performance on experimentally stressed woody plants: a meta-analysis. *Annu. Rev. Entomol.* 43:195–216.
- Krokene, P. and H. Solheim. 1999. What do low density inoculations with fungus tell us about fungal pathogenicity and tree resistance? *In Physiology and Genetics of Tree–Phytophage Interactions*. Eds. F. Lieutier, W.J. Mattson and Wagner. INRA Editions, Les Colloques No. 90, pp 353–362.
- Långström, B., C. Hellqvist, A. Ericsson and R. Gref. 1992. Induced defence reaction in Scots pine following stem attacks by *Tomicus piniperda*. *Ecography* 15:318–327.
- Långström, B., H. Solheim, C. Hellqvist and R. Gref. 1993. Effects of pruning young Scots pines on host vigour and susceptibility to *Leptographium wingfieldii* and *Ophiostoma minus*, two blue stain fungi associated with *Tomicus piniperda*. *Eur. J. For. Pathol.* 23:400–415.
- Lieutier, F. 1995. Associated fungi, induced reaction and attack strategy of *Tomicus piniperda* (Coleoptera: Scolytidae) in Scots pine. *In Behavior, Population Dynamics and Control of Forest Insects*. Eds. F.P. Hain, S.M. Salom, W.F. Ravlin, T.L. Payne and K.F. Raffa. Ohio State University, OARDC, Wooster, USA, pp 139–151.
- Lieutier, F. and J. Léveux. 1985. Les relations conifères-scolytes: importance et perspectives de recherches. *Ann. Sci. For.* 42:359–370.
- Lieutier, F., A. Yart, J. Garcia, M.C. Ham, M. Morelet and J. Léveux. 1989. Champignons phytopathogènes associés à deux coléoptères Scolytidae du Pin sylvestre (*Pinus sylvestris* L.) et étude préliminaire de leur agressivité envers l'hôte. *Ann. Sci. For.* 46:201–216.
- Lieutier, F., A. Yart, J. Garcia and M.C. Ham. 1990. Cinétique de croissance des champignons associés à *Ips sexdentatus* Boern et *Tomicus piniperda* L. (Coleoptera: Scolytidae) et des réactions de défense des pins sylvestres (*Pinus sylvestris* L.) inoculés. *Agronomie* 10:243–356.
- Lieutier, F., J. Garcia, P. Romary, A. Yart, H. Jactel and D. Sauvard. 1993. Inter-tree variability in the induced defence reaction of Scots pine to single inoculations by *Ophiostoma brunneo-ciliatum*, a bark-beetle associated fungus. *For. Ecol. Manage.* 59:257–270.
- Lombardero, M.J., M.P. Ayres, P.L. Lorio and J.J. Ruel. 2001. Environmental effects on constitutive and inducible resin defences of *Pinus taeda*. *Ecol. Lett.* In press.
- Lorio, P.L. 1986. Growth-differentiation balance: a basis for understanding southern pine beetle-tree interactions. *For. Ecol. Manage.* 14:259–273.
- Lorio, P.L. and R.A. Sommers. 1986. Evidence for competition for photosynthates between growth processes and oleoresin synthesis in *Pinus taeda* L. *Tree Physiol.* 2:301–306.
- Lorio, P.L., M.S. Frederick and T.D. Paine. 1995. Environment and ontogeny modify loblolly pine response to induced acute water deficits and bark beetle attack. *For. Ecol. Manage.* 73:97–110.
- Mencuccini, L. and J. Grace. 1996. Developmental patterns of above-ground hydraulic conductance in Scots pine (*Pinus sylvestris* L.) age sequence. *Plant Cell Environ.* 19:939–946.
- Morelet, M. 1988. Observations sur trois Deutéromycètes inféodés aux pins. *Ann. Soc. Sci. Nat. Var.* 40:41–45.
- Newbanks, D., A. Bosch and M.H. Zimmermann. 1983. Evidence for xylem dysfunction by embolization in Dutch elm disease. *Phytopathol.* 73:1060–1063.
- Paine, T.D., K.F. Raffa and T.C. Harrington. 1997. Interactions among scolytid bark beetles, their associated fungi and live host conifers. *Annu. Rev. Entomol.* 42:179–206.
- Raffa, K.F. and A.A. Berryman, 1983. Physiological aspects of lodgepole pine wound responses to a fungal symbiont of the mountain pine beetle, *Dendroctonus ponderosae* (Coleoptera: Scolytidae). *Can. Entomol.* 115:723–734.

- Reeve, J.R., M.P. Ayres and P.L. Lorio. 1995. Host suitability, predation and bark beetle population dynamics. *In* Population Dynamics: New Approaches and Synthesis. Eds. N. Cappucino and P. Price. Academic Press, San Diego, CA, pp 339–357.
- Reich, P.B. and T.M. Hinckley. 1989. Influence of pre-dawn water potential and soil-to-leaf hydraulic conductance on maximum daily diffusive conductance in two oak species. *Funct. Ecol.* 3:719–726.
- Solheim, H. and B. Långström. 1991. Blue-stain fungi associated with *Tomicus piniperda* in Sweden and preliminary observations on their pathogenicity. *Ann. Sci. For.* 48:149–156.
- Solheim, H., B. Långström and C. Hellqvist. 1993. Pathogenicity of the blue-stain fungi *Leptographium wingfieldii* and *Ophiostoma minus* to Scots pine: effect of tree pruning and inoculum density. *Can. J. For. Res.* 23:1438–1443.
- Sperry, J.S., J.R. Donnelly and M.T. Tyree. 1988. A method for measuring hydraulic conductivity and embolism in xylem. *Plant Cell Environ.* 11:34–40.
- Stephen, F.M. and T.D. Paine. 1985. Seasonal patterns of host tree resistance to fungal associates of the southern pine beetle. *Z. Angew. Entomol.* 99:113–122.
- Stephen, F.M., T.D. Paine and M.P. Lih. 1983. Understanding bark beetle/host interactions: a means for improving decision strategies. *Z. Angew. Entomol.* 96:257–265.
- Tyree, M.T. and J.S. Sperry. 1989. Vulnerability of xylem to cavitation and embolism. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 40:19–38.
- Waring, G.L. and N.S. Cobb. 1992. The impact of plant stress on herbivore population dynamics. *In* Insect–Plant Interactions, Vol. 4. Ed. E.A. Bernays. CRC Press, Boca Raton, FL, pp 167–226.
- Wei, C., E. Steudle and M.T. Tyree. 1999. Water ascent in plants: do ongoing controversies have a sound basis? *Trends Plant Sci.* 4:372–375.
- Zimmermann, M.H. 1983. Xylem structure and the ascent of sap. Springer-Verlag, Berlin, 143 p.